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Control of Hypertrophic Scar Growth Using Selective Photothermolysis

Steven R. Reiken, PhD,[1] Sean F. Wolfort, MD,[1] Francois Berthiaume, PhD,[1] Carolyn Compton, MD, PhD,[2] Ronald G. Tompkins, MD, ScD,[1] and Martin L. Yarmush, MD, PhD[1]*

[1]Surgical Services, Massachusetts General Hospital, Boston, Massachusetts 02114
[2]Center for Engineering in Medicine, Massachusetts General Hospital, Boston, Massachusetts 02114

Background and Objective: **Previous studies have shown a clinical improvement of hypertrophic scars (HS) after treatment with a pulsed dye laser. The objective of this study was to investigate the effects of variations in pulse wavelength and energy density on HS tissue using human HS implanted in athymic mice.**
Study Design/Materials and Methods: **Small pieces (~1 mm^3) of HS tissue were implanted into athymic mice and allowed to grow for 5 days. The implant site was then exposed to a single 450 μs pulse, and implant growth and histology were monitored for an additional 12 days. Laser wavelength and energy density ranges tested were 585–600 nm and 2–10 J/cm^2, respectively.**
Results: **Using a wavelength of 585 nm, laser treatment inhibited implant growth by 70% at 6 J/cm^2 and 92% at 10 J/cm^2, respectively. The inhibitory effect decreased as the laser wavelength was increased from 585 to 600 nm. A widespread destruction of the implant microvasculature with a minor effect on surrounding extracellular matrix at the highest light dose were observed.**
Conclusion: **Pulsed laser treatment inhibits HS implant growth in nude mice. This effect is likely mediated by selective photothermolysis of the implant microvasculature. Lasers Surg. Med. 21:7–12, 1997.** **© 1997 Wiley-Liss, Inc.**

Key words: hypertrophic scar; microvasculature; pulsed dye laser

INTRODUCTION

Hypertrophic scar (HS) formation is a major cause of functional and cosmetic deformity after burn injury. Pharmacological treatment (e.g., steroid injection), compression garments, and surgical excision followed by skin grafting are the therapies currently used to prevent and treat HS [1]. Although they often ameliorate HS, they are severely limited in their ability to restore normal skin function and appearance, and HS will often recur after treatment.

Preliminary studies by Alster et al. [2, 3] suggest that treatment of HS with a 585 nm pulsed dye laser can lead to a significant clinical improvement. In these studies, the altered skin surface texture was thought to result from the elimination of blood vessels by the treatment since the light pulses are known to be primarily absorbed by hemoglobin and cause blood coagulation in normal skin [4–6]. Although these results were encouraging, little analysis was performed, and no attempt was made to evaluate the effect of varying the irradiation parameters on HS growth.

In this study, we selected an athymic mouse implant model to study the effect of laser wavelength and energy density in the treatment of HS. Previous studies with this model have shown its utility for testing different therapeutic strategies for the control of HS [7–11]. Implants grow in vol-

*Correspondence to: Martin L. Yarmush, Shriners Burns Institute Research Center, One Kendall Square, Building 1400W, Cambridge, MA 02139.

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ume for ~ 2 weeks while maintaining their histological appearance, cellularity, and glycosaminoglycan distribution. To test the potential of selected photothermolysis (SPT) for treating HS, implants were treated with a pulsed laser using different combinations of laser wavelengths and energy densities. Scar growth was monitored for 10 days following irradiation. The results clearly indicate that SPT treatment was able to effectively inhibit HS implant growth and caused a marked destruction of the microvasculature with little effect on surrounding tissue.

MATERIALS AND METHODS

Mature scars ranging from 1–3 years in age from three different burn patient donors were used in this study. After surgical excision, the tissue samples were minced into 1×1×1 mm cubes (measured using a metric ruled scalpel handle) and implanted into athymic mice using previously described techniques [see 11 for details].

Each animal received two implants. Five days after implantation, one of the implants was treated with a single pulse from a flash lamp-pumped pulsed dye SPTL-1 laser (Candela, Wayland, MA); the other implant was shielded with a dark cloth (see Fig. 1). The volume of each implant at day 0 was assumed to be equal to the preimplant volume of 1.0 mm^3. Implant volumes were then assessed at intervals of 3–4 days for up to 12 days using calipers to measure the two-dimensional area (D1 × D2) and thickness (D3) of the implants and the volumes were calculated, as described previously [11], using the formula for an ellipsoid ($\pi/6 \times D1 \times D2 \times D3$). Data were analyzed using ANOVA to determine statistical significance between the mean volumes of the laser-treated and untreated implants at various time points after treatment.

Two sets of experiments were conducted. First, a total of 18 animals were implanted with HS tissue using the tissue from two donors. The laser wavelength was kept at 585 nm, whereas the laser energy densities tested were 2.0 J/cm^2 (6 animals), 6.0 J/cm^2 (6 animals), and 10 J/cm^2 (6 animals). In addition, the effect of varying the laser wavelength from 585 nm to 600 nm on implant growth was tested using a constant energy density of 6 J/cm^2. Twelve animals were implanted using tissue from a single donor in this set of experiments.

Histologic analysis was performed on three animals in each group following treatment of implants at a wavelength of 585 nm and energy densities of 2 J/cm^2, 6 J/cm^2, and 10 J/cm^2. At 24 hours postlaser treatment, the skin implants were excised and immediately fixed in 10% formalin. Samples were embedded in paraffin, sectioned, and stained with hematoxylin-eosin.

RESULTS

Effect of Pulsed Laser on Implant Growth

We first characterized the effect of laser energy density on implant growth as shown in Figure 2. Implants treated with 2 J/cm^2 displayed normal growth. However, implants treated with 6 J/cm^2 and 10 J/cm^2 were 70% and 92% smaller than untreated controls at day 17, respectively. These latter differences were found to be statistically significant.

The effect of laser wavelength on implant growth is shown in Figure 3. Maximum growth inhibition occurred at a wavelength of 585 nm, resulting in an implant size 81% smaller than the untreated control ($P < 0.001$). Significant inhibition of growth was also observed in implants treated with laser wavelengths of 590 nm (72%, $P < 0.001$) and 595 nm (65%, $P < 0.001$), but minimal inhibition was observed with 600 nm pulses.

Effect of Pulsed Laser Treatment on Implant Histology

Twenty-four hours following pulsed laser treatment at an energy density of 2 J/cm^2, no histopathological changes were observed in the implants (Fig. 4A). Vessels were patent and thin-walled. Endothelial cells appeared uninjured. Endothelial cell size and staining properties were within normal limits, and no discernible morphological changes were seen in the stroma surrounding vessels. At a laser energy density of 6 J/cm^2, vessels throughout the implant appeared mildly thickened (Fig. 4B). Endothelial cells were mildly swollen and slightly hyperchromatic, and rare necrotic cells were seen within vessel lumens. No tinctorial or morphological changes of the perivascular stroma were apparent. When a laser energy density of 10 J/cm^2 was used, vessel walls throughout the implant were markedly thickened 24 hours posttreatment, and their lumens contained collections of cellular debris (Fig. 4C). At higher magnification (Fig. 4D), the perivascular collagen appeared vacuolated and basophilic, a histologic appearance consistent with denaturation. Widespread and severe endothelial injury

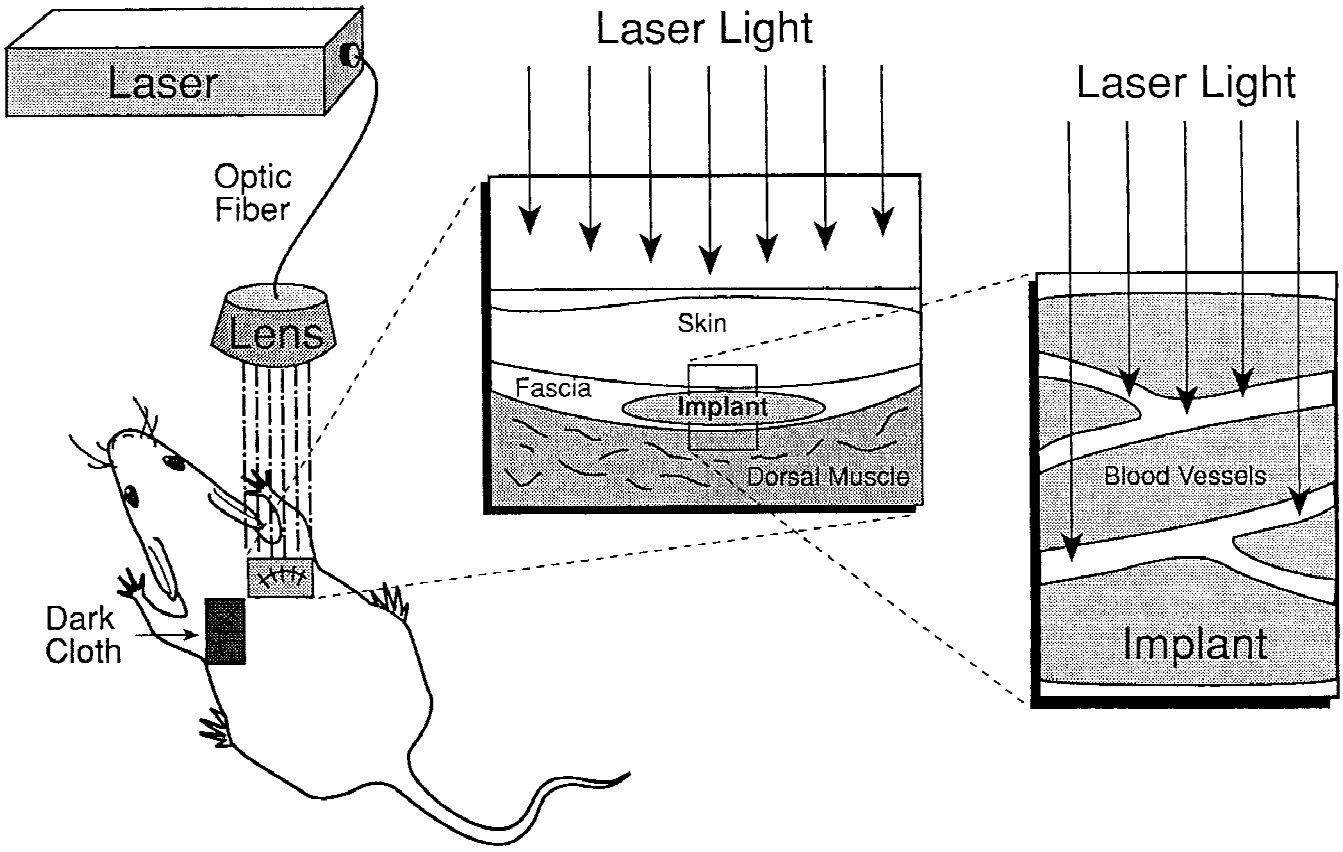

Fig. 1. Schematic diagram of pulsed laser treatment of HS implants. De-epithelialized HS tissue was cut into 1 mm^3 pieces and implanted subcutaneously in the shoulder of nude mice. Each animal received two implants. After allowing the implants to grow for 5 d, one implant site was exposed to a single laser light pulse; the other site was covered by a dark cloth to serve an an untreated control.

was apparent, with marked cellular swelling and endothelial nuclear fragmentation.

Implants treated with 6.0 J/cm^2 and histologically evaluated 48 hours, 72 hours, and 10 days posttreatment (data not shown) were less vascular compared to untreated implants. No other morphological differences were observed between treated and untreated implants. In all cases, the histopathological findings appeared to be uniform over the entire cross section of the implant.

DISCUSSION

In this study, the effects of variations in pulse wavelength and energy density on the inhibition of the growth of HS implants in athymic mice were evaluated. No growth inhibition was observed in implants treated with 2 J/cm^2; however, significant growth inhibition did occur at fluences of 6 J/cm^2 and 10 J/cm^2. Also, within the parameters examined, maximal inhibition was observed when the energy absorbed by the oxyhemoglobin in erythrocytes was the highest (585 nm in this study) and minimal inhibition occurred when the absorbance of oxyhemoglobin was minimal (600 nm).

Histologic evaluation of the implants was done primarily at 24 hours postlaser treatment because previous research with the pulsed laser [5] demonstrated significant vessel wall necrosis within this time period. The most significant finding from the histology was that the extent of the inhibition of growth correlated with the extent of vascular damage, with no vascular damage seen at 2 J/cm^2 and the maximal effect seen at 10 J/cm^2. In addition, no morphological changes other than vascular damage was observed in implants treated with 6.0 J/cm^2 indicating that the growth was not due to necrosis of the implanted material.

In a recent report, Alster reported clinical

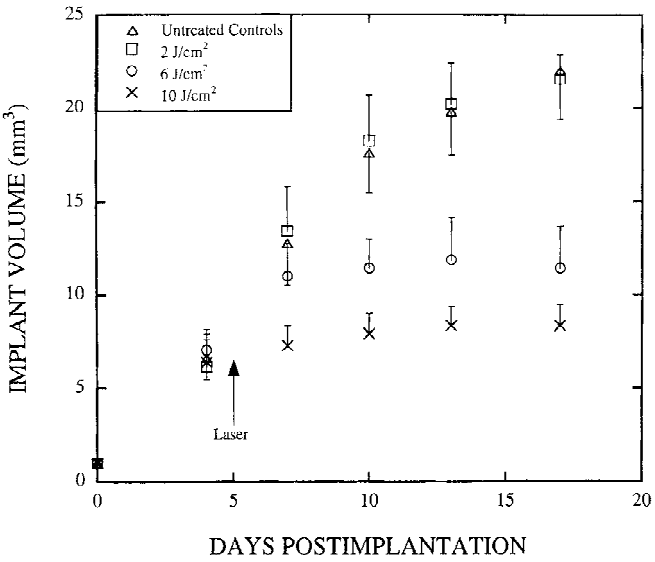

Fig. 2. Effect of laser energy density on HS implant growth. HS tissue was implanted (day 0) as 1 mm^3 cubes. At day 5 postimplantation, one implant site was treated with the pulsed laser. Laser wavelength was kept constant at 585 nm. Laser energy densities tested were 2 J/cm^2 (n = 6), 6 J/cm^2 (n = 6), and 10 J/cm^2 (n = 6). Implant volume (mean ± SD) vs. time is shown for laser-treated implants and pooled untreated controls. The reduction in HS implant growth was significant with laser energy densities of 6 J/cm^2 ($P<0.005$ at day 17) and 10 J/cm^2 ($P<0.001$ at day 17).

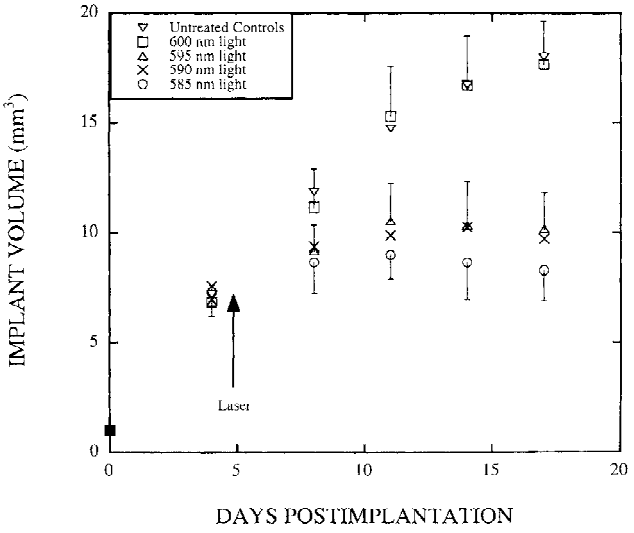

Fig. 3. Effect of laser wavelength on HS implant growth. At day 5 postimplantation, one implant site was treated with the pulsed laser. Laser energy density was kept constant at 6 J/cm^2. Laser wavelengths tested were 585 nm (n = 4), 590 nm (n = 4), 595 nm (n = 4), and 600 nm (n = 4). Implant volume (mean ± SD) vs. time is shown for laser-treated implants and pooled untreated controls. The extent of inhibition of HS implant growth increases with decreasing wavelength, and is statistically significant at wavelengths of 595 nm ($P<0.001$ at day 17), 590 nm ($P<0.001$ at day 17), and 585 nm ($P<0.001$ at day 17).

improvement of HS in 14 patients treated with one to two pulsed laser treatments [2]. In no cases did the scars worsen due to the laser treatments and no other negative consequences were observed. The entire scar in each patient was exposed to 450 μs pulses of 585 nm light with a fluence between 6.5 and 6.75 J/cm^2. These fluences have been shown to selectively target (minimal epidermal damage) the vasculature in port wine stains (PWS) [12–14]. However, it is uncertain whether these parameters represents the optimum treatment protocol for HS. Target vessels in PWS lie approximately within 0.5 mm for young children and 1.0 mm in adults from the dermo-epidermal junction [15], whereas HS are usually several mm thick [16]. To increase the depth of the vascular damage, it is possible to increase the fluence and/or use longer wavelengths than 585 nm [17]. We observed some damage to collagen when implants were treated with an energy density of 10 J/cm^2. Other studies in human PWS also using 585 nm light have reported, in some cases, dermal collagen coagulation at 7.5–8 J/cm^2 [18]. Thus energy density cannot be significantly increased at 585 nm without imparting some nonspecific damage. However, we have reported the same extent of inhibition of growth at 585, 590, and 595 nm. Given that the depth of penetration of the laser may increase from 585 nm to 595 nm [17], it is possible that deeper damage may be imparted while keeping the energy density at 6 J/cm^2.

Because 585 nm light pulses are thought to be primarily absorbed by oxyhemoglobin, the effect of the laser of HS is likely due to a direct effect of the laser on blood vessels [2]. Examination of laser-treated HS implant tissue sections clearly suggests that the primary target of the laser is indeed the microvasculature, which may cause growth inhibition by destroying the nutrient supply to the tissue. Another possible explanation is that endothelial cells release factors in the implant that are important stimuli for its growth. Prior studies have found high levels of collagen and transforming growth factor β-1 messenger RNA in fibroblasts and endothelial cells in close proximity of blood vessels in HS and keloids [19, 20]. Thus laser-induced destruction of the microvasculature may be a way to abrogate the source of these factors and cause the resolution of

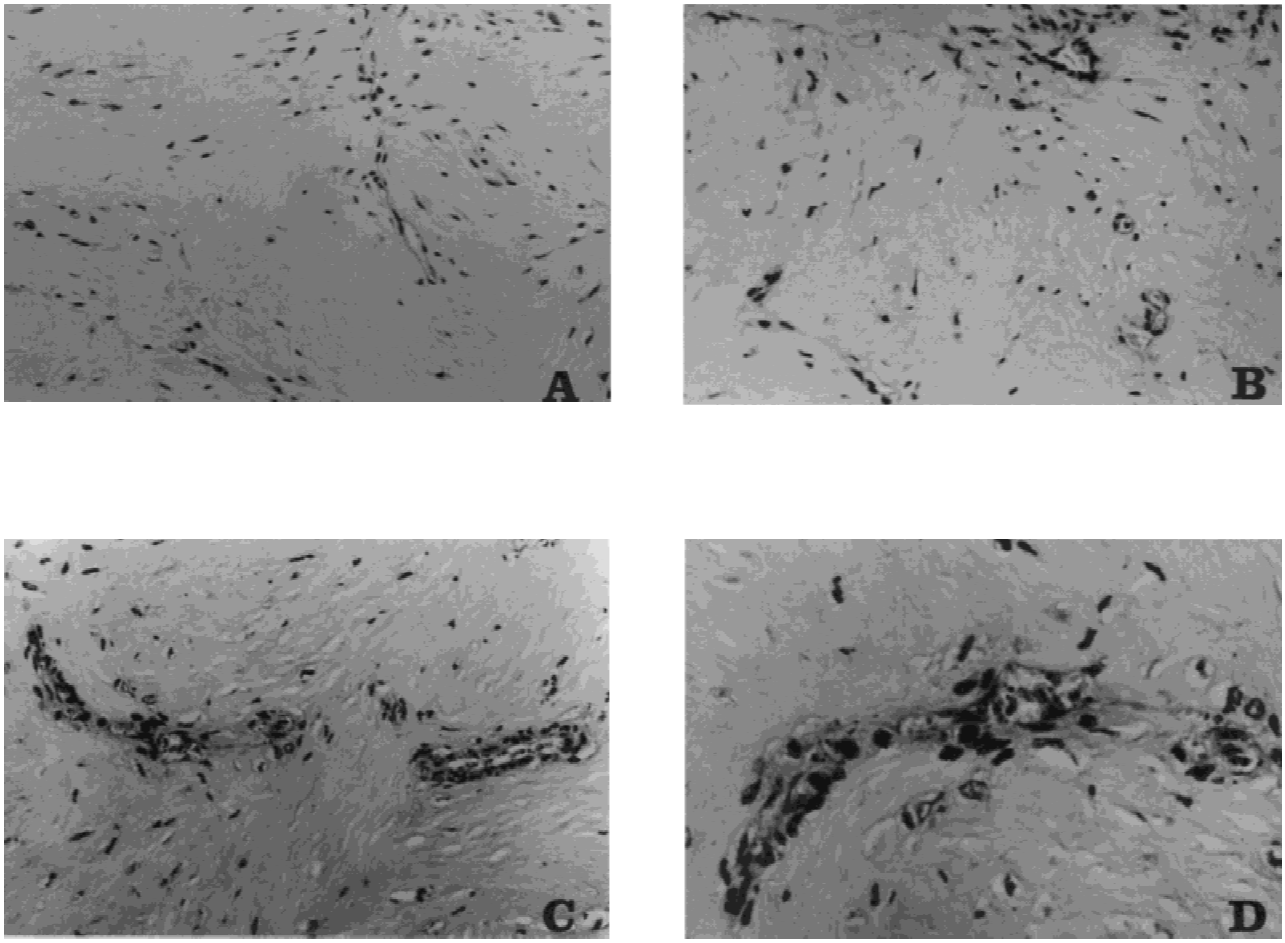

Fig. 4. Histologic appearance of HS implants treated with the pulsed laser. Implants were treated with the pulsed laser at day 5 postimplantation, and tissues harvested and stained with hematoxylin and eosin 24 h later. **A.** Implant treated with 2 J/cm^2. All vessels are thin-walled, patent, and uninflamed. Endothelial cells show no histopathologic evidence of injury, and no structural or tinctorial changes in the perivascular stroma are seen (×55; H&E). **B.** Implant treated with 6 J/cm^2. Vessel walls appear mildly thickened and endothelial cells are mildly swollen. (×55; H&E). **C.** Implant treated with 10 J/cm^2. Vessel walls are markedly thickened, and their lumen contain cellular debris. On higher magnification (**D**), the perivascular collagen appears basophilic and vacuolated, consistent with local denaturation. Widespread endothelial injury is seen with severe swelling and nuclear fragmentation. C: ×55; D: ×135; H&E).

established HS. Since several researchers have theorized that prolonged angiogenesis is a major contributing factor to HS formation [21, 22], it is also conceivable that pulsed laser treatment could be used early on to prevent HS formation by controlling the extent of angiogenesis within the wound. Therefore, this minimally invasive therapeutic tool may be a good candidate for treatment of HS.

CONCLUSIONS

Pulsed laser treatment inhibited the growth of HS implants in athymic mice. The extent of inhibition was proportional to the light dose from 6–10 J/cm^2 and was maximal at the lowest wavelength within the range tested (585–600 nm). The laser-mediated effect on growth was likely to be due to selective photothermolysis of the HS implant microvasculature.

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